Bonneville Power Administration
Fish and Wildlife Program FY99 Proposal

Section 1. General administrative information

Monitor and evaluate genetic characteristics of supplemented salmon and steelhead

Bonneville project number, if an ongoing project 8909600

Business name of agency, institution or organization requesting funding

National Marine Fisheries Service

Business acronym (if appropriate) NMFS

Proposal contact person or principal investigator:

Name	Robin S. Waples
Mailing Address	2725 Montlake Boulevard East
City, ST Zip	Seattle, WA 98112
Phone	(206) 860-3254
Fax	(206) 860-3267
Email address	robin.waples@noaa.gov

Subcontractors. List one subcontractor per row; to add more rows, press Alt-Insert from within this table

Organization	Mailing Address	City, ST Zip	Contact Name
Frank Orth	10900 NE 4th	Bellevue, WA	

NPPC Program Measure Number(s) which this project addresses.

4.1A.2, 4.1D, 7.2A.1, 7.2A.2, 7.2A.6, 7.3B.2 7.4D.1, 7.4L.1, 7.5B.1, 8.4A.1

NMFS Biological Opinion Number(s) which this project addresses.

Conservation Recommendation #3 from the "Biological Opinion for 1995 to 1998 Hatchery Operations in the Columbia River Basin" (April 5, 1995) stipulates that "The action agencies should conduct monitoring and evaluation studies for hatchery programs. This should assist NMFS in evaluating the effects of hatchery programs on listed and unlisted natural fish."

Consultation #383 - Biological Opinion on 1995-1998 Hatchery Operations in the Columbia River Basin. Issued 4/5/95
Expires 12/31/98. This BO addresses Lookingglass straying issue in Grande Ronde basin.

Direct take permits (and associated Biological Opinions) that call for genetic monitoring of populations included in this study

#847 - ODFW - Imnaha River Broodstock Collection, Mod. 4 Issued 6/28/96 - Expires 3/31/98

#919 - IDFG - Broodstock Collection Sawtooth Hatchery, Original Issued 6/16/94 - Expires 3/31/98

#921 - IDFG - Broodstock Collection McCall Hatchery, Mod. 2 Issued 8/2/96 - Expires 12/31/98

#1011- ODFW - Chinook Captive Broodstock Program, Mod. 1 Issued 6/20/97 -Expires 12/31/2000

Other planning document references.

If the project type is "Watershed" (see Section 2), reference any demonstrable support from affected agencies, tribes, local watershed groups, and public and/or private landowners, and cite available documentation.

Proposed Recovery Plan for Snake River Salmon: Tasks 4.1b, 4.3a; priority 1.

Subbasin.

Steelhead: Tucannon, Grande Ronde, Imnaha, Clearwater
Spring/summer chinook salmon: Grande Ronde, Imnaha, SF Salmon, MF Salmon, upper Salmon

Short description.

Monitor changes over time in genetic characteristics of hatchery, natural (supplemented), and wild (unsupplemented) populations of Snake River spring/summer chinook salmon and steelhead. Use results to help evaluate effectiveness of supplementation.

Section 2. Key words

Mark	Programmatic Categories	Mark	Activities	Mark	Project Types
X	Anadromous fish		Construction		Watershed
	Resident fish		O & M	+	Biodiversity/genetics

	Wildlife		Production		Population dynamics
	Oceans/estuaries	+	Research		Ecosystems
	Climate	X	Monitoring/eval.		Flow/survival
	Other	+	Resource mgmt		Fish disease
			Planning/admin.	X	Supplementation
			Enforcement		Wildlife habitat en-hancement/restoration
			Acquisitions		

Other keywords.

allozymes, DNA, PCR, RFLP, microsatellites, fin clips, nonlethal genetic sampling, historic scale DNA, stock identification, gene flow, introgression, effective population size, hatchery-wild interactions

Section 3. Relationships to other Bonneville projects

Project #	Project title/description	Nature of relationship
92-26-1 ODFW	Early life history Grande Ronde chinook	We coordinate genetic sampling with sampling from this study
90-052 NBS	Performance/stock productivity impacts of supplementation	We have shared steelhead samples from this study and we provide NBS results of our genetic analyses for some of their study streams
91-073 & 89-098 IDFG	Idaho natural production and evaluation, Intensive monitoring subproject; ISS	We coordinate genetic sampling with sampling from these studies
9604400 ODFW/ NPT	Grande Ronde sp. chinook captive broodstock program	We coordinate genetic sampling with collections for this program
9801001 LSRCP/ ODFW/ NPT	Grande Ronde sp. chinook captive broodstock O&M M&E	We coordinate genetic sampling with collections for this program
9606700 NMFS	Manchester captive broodstock O&M	We coordinate genetic sampling with this program

Section 4. Objectives, tasks and schedules

Objectives and tasks

Obj 1,2,3	Objective	Task a,b,c	Task
1	Collect samples	a	Conduct preseason evaluations of previous year escapements to identify optimal sampling strategy
		b	Coordinate sampling efforts to maximum extent possible with other ongoing projects
		c	Collect samples from hatchery, natural, and wild populations
2	Conduct genetic analyses	a	Perform allozyme and DNA analyses
		b	Perform quality control tests on preliminary data
3	Measure levels of genetic variability in each population	a	Quantify percent polymorphic loci, heterozygosity, number of alleles per locus
		b	Compare values in hatchery, natural, and wild populations
		c	Evaluate pattern of change in genetic variability over time
4	Estimate effective population size (Ne) and the ratio Ne/N for each population	a	Compute F, a measure of temporal change in allele frequency
		b	Compute r^2, a measure of gametic disequilibrium
		c	Use temporal and disequilibrium methods to obtain a combined estimate of Ne for each population
		d	Estimate total population size (N) based on redd counts, spawner surveys, or population enumeration
		e	Compute ratio Ne/N
5	Evaluate population genetic structure of natural and wild populations	a	Compute indices of genetic differentiation among natural and wild populations
		b	Perform hierarchical gene diversity analyses to partition genetic differences into various components
		c	Estimate levels of gene flow among populations based on genetic data

6	Evaluate genetic effects of supplementation on target and non-target populations	a	Compute indices of genetic differentiation between hatchery and natural and hatchery and wild populations
		b	Compare patterns of genetic change over time in hatchery populations with those in natural and wild populations
		c	Compare recent genetic data for Grande Ronde populations with historic (pre-supplementation) data obtained from DNA analysis of archived scales
7	Evaluate effectiveness of genetic monitoring	a	Quantify genetic differences between hatchery, natural, and wild populations
		b	Quantify sources of noise in analysis (sampling error, genetic drift)
		c	In light of a) and b), evaluate combined power of genetic markers (allozymes + DNA) to provide monitoring and evaluation information that is useful for an adaptive management approach to supplementation.

Objective schedules and costs

Objective #	**Start Date mm/yyyy**	**End Date mm/yyyy**	**Cost %**
1	8/89	9/00	10
2	12/89	6/01	60
3	3/90	9/01	5
4	6/90	9/01	5
5	3/90	9/01	5
6	3/90	9/01	5
7	3/01	3/02	10

Schedule constraints.

The major potential constraints are availability of parr for sampling from wild and natural populations. This was not a problem in 1989-94, but in 1995 and 1996 lethal collections of spring/summer chinook salmon for allozymes were suspended because of record low returns of adults in 1994 and 1995. In those years, we placed more emphasis on non-lethal fin clips for DNA analysis and continued to sample from hatchery populations as feasible. Lethal sampling in 1997 was restricted to three collections on the South Fork Salmon River. Sampling in 1998 and 1999 should be feasible without undue risk to wild/natural populations, but the abundance of parr in subsequent years is more uncertain. Appropriate levels of sampling will be determined in consultation with state agency biologists, and through the process of securing state and federal ESA collection permits. The recent ESA listing of Snake River steelhead will require an ESA permit for this species.

Completion date.

The study, which began in late 1989, was designed to run for at least 10 years, or about 3 salmon generations. Following completion of work in FY2000 will be a time for full review and evaluation of results obtained through that time.

In the table above, the objectives are shown to run through FY2000. At that time, results may indicate the program should continue at a similar or different scale. In the table below we have indicated tentative costs for outyears beyond FY2000 in brackets.

Section 5. Budget

FY99 budget by line item

Item	**Note**	**FY99**
Personnel		74,200
Fringe benefits		32,100
Supplies, materials, non-expendable property		48,500
Operations & maintenance		3,000
Capital acquisitions or improvements (e.g. land, buildings, major equip.)		0
PIT tags	# of tags:	0
Travel		7,000
Indirect costs		45,700
Subcontracts	Laboratory technician	39,000
Other		0
TOTAL		249,500

Outyear costs

Outyear costs	**FY2000**	**FY01**	**FY02**	**FY03**
Total budget	250,000	[250,000]	[250,000]	[250,000]
O&M as % of total	1.2	1.2	1.2	1.2

Section 6. Abstract

This genetic monitoring program is designed to evaluate the effects of outplanting hatchery-reared fish on natural and wild populations of spring/summer chinook salmon (*Oncorhynchus tshawytscha*) and steelhead (*O. mykiss*) in the Snake River Basin. The two major goals are 1) to evaluate the nature and extent of genetic changes in hatchery stocks to be used for outplanting, and 2) to quantify the genetic impact of outplanting on targeted natural stocks and non-targeted wild stocks. These goals address important components of the Fish and Wildlife Program, the proposed Snake River Recovery Plan, and other regional planning documents. Information developed in this study will also provide valuable insight into the spatial and temporal scale of genetic structure in natural and wild populations.

The basic study plan includes eight different supplementation programs (two for each species). Yearly samples are taken from the hatchery stocks used for supplementation and selected natural and wild populations. Samples are analyzed for a suite of protein and DNA characters, and data obtained will be used to test the hypothesis that the outplanted stock has no significant genetic impact on natural or wild populations. Allele frequency change over time will be used to estimate effective population size and rates of inbreeding. Avoiding deleterious effects of inbreeding is of considerable importance, particularly in stocks used in attempts to establish self-sustaining natural populations.

In addition to monitoring the effects of the individual enhancement programs, the study will provide a broader perspective of the kind of results to be expected from different methods of supplementation. Results thus should be of general use in planning and implementing enhancement programs throughout the Columbia River Basin.

Section 7. Project description

a. **Technical and/or scientific background**.

In spite of concerted management efforts, the abundance of most Pacific salmon species (*Oncorhynchus* spp.) has been substantially below historical levels in recent years (e.g., Fraidenburg and Lincoln 1985; Nehlsen et al. 1991; NRC 1996). The Columbia River Basin Fish and Wildlife Program has an interim goal of doubling the abundance of anadromous salmonids in the Columbia River Basin. The program calls for improvements in a variety of areas, including mainstem passage, habitat restoration, and control of disease, but an important component of the program is supplementation--that is, the use of artificial propagation to increase the abundance of naturally-spawning salmon and steelhead (*O. mykiss*). A number of supplementation programs are already under way throughout the basin.

The most recent review of supplementation research (Miller et al. 1990) indicates that there are still substantial gaps in our knowledge of how to supplement natural populations effectively. Among the most important, yet least understood, factors to consider are the genetic consequences of releasing hatchery-reared fish into the wild. This is an important consideration because the genetic makeup of native wild stocks was presumably shaped by hundreds or thousands of years of adaptation to local conditions. Transplanted fish may be less well suited to local conditions, and hybridization may cause a reduction in fitness of the native stock through outbreeding depression. Emlen (1991) reviewed some of the evidence for outbreeding depression in other organisms and suggested a model that may be applicable to Pacific salmon. These possibly adverse effects can be reduced by using a stock for outplanting that is genetically similar to the local stock. However, unless the hatchery stock used for outplanting is genetically identical to the natural stock being supplemented, a successful supplementation program will entail some genetic change to the local stock. It is important, therefore, to have a means of assessing the nature and extent of genetic changes that occur as a result of supplementation.

Unfortunately, traditional monitoring methods are not well suited to determining whether outplanted fish are having any permanent genetic effect on the target stock. Physical tags may indicate whether a fish returns as an adult, but not whether it produces offspring that survive and contribute to subsequent generations. It is possible, for example, to release large numbers of juvenile fish in a stream over a period of many years and, in the end, not know whether 1) the natural population has been entirely replaced, 2) the current population contains genetic material from both the original population and the outplanted fish, or 3) the outplanted fish have had no permanent genetic impact on the natural population (Fig. 1). Hindar et al. (1991) reviewed data from a number of studies of salmonids that show each of these outcomes is possible.

A genetic monitoring program provides the best opportunity for determining which of these scenarios has occurred. Because genetic markers are heritable, they reveal information about the reproductive success of transplanted fish and the degree to which the native and transplanted gene pools have been integrated. Furthermore, the same approach can be used to evaluate the genetic effects of outplants on nearby wild stocks that are not intended to be supplemented.

Specific reference to goals and objectives of the 1994 Fish and Wildlife Program and other research directives are made below in Section 7c.

b. Proposal objectives.

Objective 1. Collect samples

Based on preseason surveys of juvenile distribution and redd counts from the previous year, collections will be arranged, coordinating wherever possible with other field activities. Samples will include hatchery, natural, and wild collections representing the study sites in different basins.

Assumption:

Sampling is random with respect to the entire population. Again, some departures from strict randomness are expected, but non-representative samples can bias results. Collection methods attempt to minimize possibilities of collecting a non-representative sample, but randomness is difficult to guarantee in field collections of juveniles. In some cases, a sample of progeny from a relatively few individuals can be identified by an unusually low estimated ratio of effective to total population size.

Objective 2. Conduct genetic analyses

Preliminary analyses of allozyme and DNA data will be conducted to assure its integrity and identify any potential errors or sampling anomolies.

Testable hypothesis:

Genotypic frequencies do not differ from those expected under Hardy-Weinberg equilibrium

Assumption:

Allozyme variation is largely neutral. Undoubtedly some departures from strict neutrality exist, but substantial departures might bias conclusions drawn from the data. With respect to temporal variation, this assumption can be tested as described below under "testable hypotheses."

Objective 3. Measure levels of genetic variability in each population

Genetic variability within populations will be evaluated in a number of different ways. Comparisons of variability in hatchery, natural, and wild populations will be made and changes in levels of variability will be evaluated through time.

Testable hypotheses:

Levels of genetic variability are the same in hatchery, natural, and wild populations

Levels of genetic variability do not change over time

Objective 4. Estimate effective population size (Ne) and the ratio Ne/N for each population

Fixation indices and gametic disequilibrium will be used to estimate and evaluate the relationship between effective population size and census size (N) estimated from redd counts, spawner surveys, and population enumeration.

Testable hypotheses:

Inter-locus variance of F (a measure of allele frequency change over time) is no larger than would be expected if all changes are due to sampling error and genetic drift.

The relationship between Ne and N is the same in hatchery and natural populations.

The relationship between wild Ne and N in natural/wild populations is the same in years of high and low escapements.

Objective 5. Evaluate population genetic structure of natural and wild populations

Fixation indices and hierarchical gene diversity analyses will be used to partition genetic variation into spatial and temporal components. These relationships will be used to estimate levels of gene flow among populations.

Testable hypotheses:

There are no genetic differences among natural populations, except those that can be attributed to sampling error and random year-to-year variation.

Genetic affinities among geographic populations change randomly over time

Levels of gene flow among populations are less than X individuals per generation

Objective 6. Evaluate genetic effects of supplementation on target and non-target populations

Indices of genetic differentiation will be calculated between hatchery and natural, and hatchery and wild populations. Patterns of genetic change will be examined through time in the three classes of populations. In the Grande Ronde basin, short-term change over the period of 10 years represented by this study will be compared to change seen since the inception of the Lookingglass Hatchery program.

Testable hypotheses:

There are no genetic differences between hatchery populations and natural populations they were derived from.

Populations that have been supplemented show the same magnitude of genetic change over time as unsupplemented populations.

Non-target wild populations have not been genetically affected by hatchery strays.

Current natural populations in the Grande Ronde basin are not more similar to the Rapid River stock from Lookingglass Hatchery than they were historically.

Populations in which genetic effects of supplementation can be detected show the same patterns of abundance and productivity as unsupplemented populations.

Objective 7. Evaluate effectiveness of genetic monitoring

An overall assessment will be made of the power of genetic markers to provide monitoring and evaluation information that is useful for an adaptive management approach to supplementation. We already know that this approach can be very useful in some instances and less useful in others, but an overall evaluation has been deferred until the end of the study because such extensive progress has been made in developing DNA markers that an assessment any earlier would be premature.

Testable hypothesis:

Genetic differences among populations are so small and temporal variation so great that relationships among samples, and effects of supplementation, cannot be meaningfully evaluated

Note: This general hypothesis can be tested for each supplementation program in each of the species (8 tests altogether). If the hypothesis is rejected, then we can evaluate power of the combined genetic data (allozymes + DNA) to detect genetic differences of various magnitudes. Taken as a whole, these results should provide considerable insight into the general usefulness of genetic monitoring and evaluation programs.

c. Rationale and significance to Regional Programs.

The central tenet of adaptive management, as framed in the 1987 Columbia River Basin Fish and Wildlife Program, is maintaining flexibility to respond to biological indicators of the success or failure of specific management strategies. This flexibility, however, is of little use without an adequate monitoring and evaluation program to provide the basis for making scientifically-based decisions. Supplementation is an experimental strategy that has considerable promise but also many associated uncertainties. The genetic consequences of supplementing natural populations with hatchery reared fish are among the biggest uncertainties, and this issue cannot be addressed without a monitoring program that focusses on genetic markers. This study is thus an essential component of a more comprehensive, cross-disciplinary monitoring and evaluation program for salmon supplementation.

Upriver stocks of chinook salmon and steelhead were given highest priority for research in the FWP. The proposed research directly addresses a number of goals in the revised (1994/95) Program, including 4.1A.2 (which indicates that “program activities should pose no appreciable risk to biological diversity among or within fish populations” and “activities should be followed-up with monitoring and evaluation”); 4.1D (calling for establishment of Biological Diversity Performance Standard that “will be the existing level of biological diversity”); 7.2A.1, parts 1) and 2) (which call for “coordination of hatchery production to reduce [genetic and ecological] impacts of hatchery stock on wild and naturally spawning fish” and “monitoring and evaluation of hatchery and wild and naturally spawning stock interactions”); 7.2A.6 (stating the “goal of increasing sustained production while maintaining genetic resources,” “avoiding adverse genetic effects on wild, natural and hatchery fish populations,” and “maintenance of genetic integrity (including...effective population size)”); 7.3B.2 (which provides for implementation of high priority supplementation projects, including "design, construction, operation, maintenance, monitoring, and evaluation"); 7.4D.1 (calling for “development of genetically sound methods of sourcing and breeding brood stock to ensure genetic stability”); 7.4L.1 (which directs Bonneville to fund "biological monitoring and evaluation studies" for Northeast Oregon production facilities); 7.5B.1 (which identifies the need to “develop an experimental design for implementing monitoring and evaluating supplementation”); 8.4A.1 (which directs managers to “develop and implement an expanded genetic stock identification program for monitoring inriver and ocean fisheries.” The proposed research has developed and implemented new genetic markers and will continue to lay the ground work to meet this goal); 8.4B.1 (calls for evaluation of “the potential for using DNA ‘fingerprinting’ and other methods to identify chinook...and steelhead stocks in the Columbia River”).

In addition to addressing the above concerns, this study has also provided important information in a variety of areas, including 1) Population structure of Snake River spring/summer chinook salmon for use in listing determinations and recovery planning under the Endangered Species Act; 2) Effects on natural populations of straying by non-native hatchery fish in the Grande Ronde basin; 3) Effective size of wild, natural, and hatchery populations; 4) A temporal series of baseline data for comparison with data from future sampling programs; 5) Population structure of Snake River steelhead for use in the ESA status review for coastwide steelhead populations being conducted by NMFS.

Specimens used in this study have also been made available to researchers at the University of Idaho for use in attempts to identify scale pattern characters that will distinguish hatchery and wild fish and to scientists at the U.S. Fish and Wildlife Service, who are examining the incidence of bacterial kidney disease.

The genetic monitoring program will be coordinated with existing and proposed supplementation research by Washington Department of Fish and Wildlife (WDFW), Oregon Department of Fish and Wildlife (ODFW), and Idaho Department of Fish and Game (IDFG). Collections of juvenile Snake River spring/summer chinook salmon are authorized under ESA permit number 852.

d. Project history

This project has retained the same number and same essential focus since it began in 1989. Past costs have averaged $273K per year. Analysis is currently underway for samples from the 9th year of field collections. Although the central focus of the research has remained unchanged, the sampling design has been somewhat flexible to respond to high priority issues associated with supplementation in the Snake River basin that have arisen since 1989. For example, for the past several years we have conducted intensive genetic monitoring in the Grande Ronde basin to assess impacts to natural populations of straying by Rapid River stock hatchery fish. The initial study design identified the Lostine River as the only natural/wild population to be sampled in the Grande Ronde basin. Similarly, we have considerably expanded the geographic coverage of our steelhead collections within the basin to evaluate effects of outplanting non-native stocks such as Dworshak and Pahsimeroi.

Major results achieved

Important results obtained to date include the following:

1) Snake River spring/summer chinook have higher levels of genetic variability than had been suggested by previous studies;

2) Snake River steelhead populations are highly polymorphic with a large number of variable loci, which increases power to resolve stock structure and effects of supplementation;

3) Levels of genetic variability in hatchery populations do not differ substantially for those of natural and wild populations;

4) Spring/summer chinook salmon populations are spatially structured and the structure appears to be stable over time. Structure in steelhead appears to be slightly less well-defined, except in the Clearwater basin;

5) The Dworshak Hatchery population of steelhead is the most genetically distinctive population in the Snake River basin. Analysis of natural/wild populations believed to be affected by strays or outplants from Dworshak Hatchery do not show evidence of substantial genetic effects from this stock;

6) Considerable genetic diversity is found among natural/wild populations of spring/summer chinook salmon from the Grande Ronde basin. However, samples

from some streams in some years are genetically very similar to the non-native Lookingglass Hatchery stock;

7) Genetic indices suggest that gene flow among subpopulations is on the order of a few (1-2) migrants per year;

8) Indirect genetic estimates suggest that the ratio N_b/N in natural and wild populations of chinook salmon is about 0.2 - 0.4;

9) A large number of DNA markers have been developed that greatly increase our ability to monitor supplementation;

10) Preliminary work with DNA isolation from archived scale cards showed considerable promise for characterizing historic populations.

Project Reports and Technical Papers

Waples, R. S., D. J. Teel, and P. B. Aebersold. 1991. A genetic monitoring and evaluation program for supplemented populations of salmon and steelhead in the Snake River Basin. Annual Report of Research to Bonneville Power Administration, Portland, OR, 50p.

Utter, F. M., R. S. Waples, and D. J. Teel. 1992. Genetic isolation of previously indistinguishable chinook salmon populations of the Snake and Klamath Rivers: Limitations of negative data. Fish. Bull. (U.S.) 90:770-777.

Waples, R. S., O. W. Johnson, P. B. Aebersold, C. K. Shiflett, D. M. VanDoornik, D. J. Teel, and A. E. Cook. 1993. A genetic monitoring and evaluation program for supplemented populations of salmon and steelhead in the Snake River Basin. Annual Report of Research to Bonneville Power Administration, Portland, OR, 179 p.

Park, L. K., P. Moran, and R. S. Waples (editors). 1994. Application of DNA technology to the management of Pacific salmon. Proceedings of the workshop, 22-23 March 1993, Seattle, WA. U.S. Dept. Commerce, NOAA Tech. Memo. NMFS-NWFSC-17, 178 p.

Waples, R. S., and C. Do. 1994. Genetic risk associated with supplementation of Pacific salmonids: Captive broodstock programs. Can. J. Fish. Aquat. Sci. 51 (Suppl. 1):310-329.

Park, L. K., and P. Moran. 1994. Developments in molecular genetic techniques in fisheries. Reviews in Fish and Fisheries Biology 4:272 299.

Park, L. K., P. Moran, and D. Dightman. 1995. A polymorphism in intron D of the chinook salmon growth hormone 2 gene. Animal Genetics. 2(26):285.

Park, L. K., P. Moran, and D. Nickerson. 1994. Application of the oligonucleotide ligation assay (OLA) to the study of chinook salmon populations from the Snake River. In, L. K. Park, P. Moran and R. S. Waples (eds.). Application of DNA technology to the management of Pacific salmon. U.S. Dep. Commer., NOAA Tech. Memo NMFS NWFSC-17:91-97.

Park, L. K., P. Moran, and D. A. Dightman. 1996. A chinook salmon PCR-RFLP marker in the p53 locus. Animal Genetics 27:127-128.

Moran, P., D. A. Dightman, R. S. Waples, and L. K. Park. 1997. PCR-RFLP analysis reveals substantial population-level variation in the introns of Pacific salmon (*Oncorhynchus* spp.). Mol. Mar. Biol. Biotechnol. 6:318-330.

Moran, P., D. A. Dightman, L. K. Park. 1998. Nonelectrophoretic genotyping using allele-specific PCR and a dsDNA-specific dye. Biotechniques *accepted*.

Waples, R. S. 1998. Separating the wheat from the chaff: Spatial and temporal patterns of genetic differentiation in marine species. J. Heredity (in press).

Ford, M.J. 1998. Testing models of migration and isolation among populations of chinook salmon (Oncorhynchus tschawytscha). Evolution (in press).

Adaptive management implications

As discussed in section 7.c., this monitoring and evaluation research plays an integral role in adaptive management of supplementation within the Columbia River basin. Informed decisions about appropriate management actions cannot be made without detailed information about the effects of actions that have already been taken.

e. Methods.

Sampling

Yearly samples will be taken from hatchery, wild, and natural populations involved in the study. Initially samples were 100 fish per population per year, but in recent years this has been scaled back to 80 and then 60 fish per sample to minimize effects on at-risk wild and natural populations. Hatchery samples are smolts or presmolts shortly prior to release. Field samples involve parr or smolts; for steelhead, we work closely with local biologists to avoid collecting resident rainbow trout. Field collections are made with seines or electroshockers and are conducted in accordance with NMFS ESA permit #852.. Allozyme samples are frozen in the field on dry ice or liquid nitrogen and transported or shipped to Seattle for storage and analysis at

-80 ^{o}C.

Protein electrophoresis

Protein electrophoresis follows the procedures of Aebersold et al. (1987). Laboratory procedures have been standardized among the agencies participating in the Coastwide Genetic Stock Identification Consortium. In particular, we are working closely with Washington Department of Fish and Wildlife personnel to ensure that data gathered by both agencies are compatible and reflect state-of-the-art laboratory techniques.

For each fish, genotypic data will be gathered for a series of enzyme systems coding for approximately 40-60 gene loci known to be variable in chinook salmon (steelhead have comparable, or slightly higher, levels of genetic variability). The number of loci that are polymorphic in any given sample will be fewer and varies somewhat geographically, but typically will be about 20-40.

DNA methods

In recent years, the use of DNA techniques has added significantly to the repertoire of research tools available to the salmon genetics community (Park and Moran 1994) DNA markers have served to augment allozyme data, providing additional power to identify subtle differences among populations and small genetic changes through time. They also simplify field collection of tissues, because tissues can be stored and shipped at ambient temperature, rather than requiring dry ice or liquid nitrogen. Further, even small juveniles can be easily sampled nonlethally by taking small fin clips. Most importantly for this work, it is possible to sample historic populations available as archived scale collections.

In this study, two major classes of nuclear DNA markers have been developed and implemented: restriction fragment length polymorphisms (RFLPs) in the introns and other noncoding regions of nuclear genes and highly variable simple sequence repeats, or microsatellite loci. Ten intron RFLP loci and ten microsatellite loci are fully implemented for chinook; seven RFLP loci and eleven microsatellites have been implemented in steelhead. Breeding studies have been completed for many of these to verify Mendelian inheritance. Various numbers of DNA loci have been analyzed in particular populations of interest. The DNA markers showed high levels of variability among populations, and those patterns of variation were broadly concordant with allozymes.

The RFLP methods used in this study use the approach of Moran et al. (1997) to characterize allele frequency differences among populations. Briefly, Genbank sequences are used to design PCR primers that amplify introns or other noncoding sequences (typically 500 - 2000 bp in size). The amplified products are either sequenced or surveyed with restriction enzymes in a subset of individuals to find segregating sites in the populations of interest. Different alleles are represented by the presence or absence of one of more restriction sites. The microsatellite methods are similar to those presented in Olsen et al. (1996). In this case, PCR primers amplify tandem simple-sequence repeats (e.g., the DNA bases CACACA...). Allelic variation is present at these loci in the number of copies of the repeat unit and thus the size of the PCR product. Many microsatellite primer pairs are now available for Pacific salmon. In addition to developing a few loci of our own, we have taken full advantage of primers available in the research community. We continue to interact quite closely in comparing methods with other salmon research groups including Alaska Department of Fish and Wildlife, Bodega Bay Marine Laboratory, Pacific Biological Station, Washington Department of Fish and Wildlife, and especially Paul Bentzen's group at the Marine Molecular Biotechnology Laboratory, University of Washington.

During this performance period, our DNA efforts will focus on surveying larger numbers of individuals and populations for the markers we have already developed. We will take advantage of the ability to sample fin clips nonlethally to gather an unbroken temporal series of data from depressed natural populations of spring/summer chinook salmon. We will also attempt to collect historic genetic information from archived scale samples to allow a comparison of genetic profiles pre- and post-supplementation. Preliminary work with chinook salmon scale collections from other regions shows considerable promise for the use of scale archives as a viable approach for characterizing historic populations (manuscript in review). These methods should be particularly useful in evaluating the effects of the Rapid River stock hatchery program on natural population structure of chinook salmon in the Grande Ronde basin.

In the coming year, we will obtain test scale samples from NE Oregon (less valuable material collected at the same time and in the same way as the scales of interest). We will do a series of DNA extraction experiments to assure that 1) genotyping success rates are comparable to those obtained from other scale collections, and 2) that DNA can be extracted from the epithelial tissue surrounding the scale without damaging its calicified matrix. The second part of the experiment comes about in response to concerns by Rich Carmichael at ODFW that scales used for DNA analysis would not be available for microelement analysis by laser ablation. Preliminary experiments to answer this question were inconclusive.

In addition to continuing our surveys for new variable loci, we also intend to devote further effort to developing more rapid methods of conducting DNA assays. We anticipate that our previous work with ligation capture (Park et al. 1994) and allele-specific PCR (Moran et al. 1998) will lead to significantly more efficient assays and will allow examination of base substitutions not associated with any restriction site.

Data analysis

Electrophoretic phenotypes visualized on starch gels are interpreted as genotypes according to guidelines discussed by Utter et al. (1987). A chi-square test is used to compare genotypic frequencies at each variable locus in each population with frequencies expected under Hardy-Weinberg equilibrium. This test can be useful in detecting artifactual (nongenetic) variation. The method of Waples (1988) is used to evaluate genotypes and estimate allele frequencies at isoloci (duplicated gene loci). A variety of standard statistical analyses are routinely applied to the data (e.g., computing heterozygosity, gene diversity, number of alleles per locus, genetic distances, and F-statistics; testing for heterogeneity of allele frequencies among populations).

In addition to these analyses, a number of more specialized analyses are used to estimate effective population size. As the primary goal of this project is to study genetic changes over time in natural and wild populations resulting from supplementation, it is necessary to consider factors other than hatchery-wild genetic interactions that can lead to genetic change. Because supplementation is typically considered only when natural abundance is low, the effects of random genetic drift due to finite population size must be considered in evaluating observed genetic changes. Our methods for estimating effective population size include the following:

1) <u>Quantifying allele frequency change.</u> The statistic used to measure the magnitude of genetic change is $F = (P_1 - P_2)^2/[P(1-P)]$, where P_1 and P_2 are allele frequencies in samples taken at two different times and P is the mean of P_1 and P_2. F is computed for each gene locus surveyed, and a mean F over all loci in a comparison of temporally spaced samples is also computed.

2) <u>Testing for selection.</u> Although there is a body of evidence suggesting that the enzymatic gene loci sampled by electrophoresis in general are largely unaffected by natural selection, it is important to evaluate this assumption because strong selection would complicate the interpretation of changes within populations and interactions between populations. If the loci used are effectively neutral, they all should be affected by genetic drift to approximately the same degree. The method of Lewontin and Krakauer (1973) will be used to test the hypothesis that the variance of single locus F values is no larger than expected from random sampling error. A larger variance would suggest that some loci are under strong selection, and appropriate adjustments can be made in the following analyses.

3) <u>Measuring gametic disequilibrium.</u> The statistic r^2, the squared correlation of alleles at different gene loci, are computed for each pair of loci in each sample. The overall mean r^2 value is a measure of gametic disequilibrium, or non-random associations across loci.

4) <u>Estimating N_b</u>. After omitting any loci identified by the test for selection, the mean F value (computed as in #1) is used to estimate N_b, the effective number of breeders each year. The procedure follows the "temporal method" for estimating effective population size (Krimbas and Tsakas 1971; Nei and Tajima 1981; Waples 1989), as

modified specifically for Pacific salmon (Waples 1990). A general model has been developed that has been shown to be applicable to a wide range of populations with age structure like that of chinook salmon. This model is used for populations (e.g., perhaps some wild populations) for which demographic data are not available.

BecauseF is known to be distributed approximately as chi-square, confidence limits can be placed on the estimate of N_b. The mean value of r^2 provides an independent method for estimating N_b, based on the method developed by Hill (1981), and confidence limits can also be placed on this estimate. As discussed by Waples (1991), use of both temporal and disequilibrium data for the same sample can provide a more robust estimate of effective population size than is possible with either method alone. Computing the confidence limits for this combined estimate is not straightforward, but we have developed a method for identifying approximate confidence limits.

5) Estimating N. The absolute value of effective population size is important, but it is even more useful when viewed as a proportion of the total population size (N). Identifying a general relationship between N_b and N in salmon would allow extrapolation to many systems for which abundance data but no genetic data are available. In hatchery populations, N was simply the number of adults spawned each year. In natural and wild populations, we estimated N by expanding from redd counts using a factor 3.1, which has been developed for NE Oregon streams based on comparing redd counts with intensive spawner surveys (R. Carmichael, ODFW, pers. com.).

Evaluating genetic effects on natural/wild populations. Several different methods can be employed in this evaluation, depending on the type of data available. The most important question is whether pre-supplementation baseline data are available for the hatchery and natural/wild stocks involved.

a) Baseline data available. In the short term (up to about 1 generation after supplementation), the proportion of fish of hatchery and wild origin can be estimated using the mixture model of Milner et al. (1981). A variety of methods can be used to place confidence limits on the estimated contributions. In the longer term, the relative contribution of two original gene pools to a hybridized mixture can be estimated using the method discussed by Glass and Li (1953). This approach can be modified to take genetic drift into consideration (Thompson 1973).

b) Baseline data not available. Power to resolve the genetic contribution of hatchery and natural fish is reduced considerably if pre-supplementation baseline data are not available. However, the null hypothesis that the existing population represents a single gene pool (rather than a mixture of gene pools) can still be tested using gametic disequilibrium analysis. Gametic disequilibria are correlations of alleles at different gene loci, and one cause of these disequilibria is a mixture of different gene pools. Waples and Smouse (1990) showed that the power to detect mixtures of salmonid populations can be reasonably high provided that there were sufficiently large genetic differences between the stocks before mixing. This method, however, has limited power to detect mixtures involving populations that are genetically similar.

f. Facilities and equipment.

Conservation Biology's Genetics Program--the oldest fishery genetics program in the country--is well equipped and staffed to carry out the research proposed here. Since its inception in the 1970's, the Genetics Program has played a central role in the development of Pacific salmon genetics research on the West Coast. In cooperation with other agencies the Genetics Program has helped build coast-wide genetic data bases for all the North American Pacific salmon species. In addition to embracing the commonly used methods of population genetic research in salmon, the Genetics Program has pioneered new methods and new classes of markers in both DNA and allozyme analysis. With four PhD-level geneticists, this group has the experience, training, creativity and vision to take on large-scale long-term projects such as the genetic monitoring research proposed here.

Protein Genetics Laboratory

The protein genetics laboratory is a newly remodeled, fully equipped, state of the art facility for allozyme population genetic data collection. The laboratory is equipped with 15 power supplies to meet a demanding gel running schedule, a fume hood and balance weigh station for the proper use and handling of hazardous chemicals, two personal computers for recording the allozyme genetic data and for keeping track of the samples being processed, a digital camera for preserving the genetic results, as well as a number of standard laboratory items such as balances, refrigerator, freezer, centrifuge, dish washer, pH meter, and water bath. In addition, the laboratory has seven ultra cold freezers for proper storage of the samples to be analyzed. The laboratory is staffed by two full time technicians and a laboratory manager.

DNA Laboratory

The DNA laboratory is a fully equipped, state of the art facility for molecular genetic R&D and population genetic data collection. In addition to standard molecular genetic laboratory equipment (pipets, pan balances, pH meter, microcentrifuges, conventional and ultra-cold freezers, and PC and Macintosh computers), the following instrumentation and resources (with the exception of the UV plate reader) are available for our exclusive use (i.e., not a shared core facility).

Automated Fluorescent DNA sequencers--We have two of these specialized instruments, which are designed to both sequence DNA and to perform DNA fragment analysis. Although many of our methods use fluorescent labelling, we also routinely use autoradiography for both DNA sequencing and microsatellite analysis.

Ultraviolet/Visual Plate Reader--This instrument is readily available and is used by our staff for rapid and accurate quantification of DNA isolates and for assays involving the presence/absence of a PCR product (e.g., allele-specific PCR).

Thermal cyclers--We have five PCR machines for various specialized purposes, including two conventional block machines, two forced air ovens, and a thermal gradient machine for optimizing PCR conditions.

High-speed refrigerated tabletop centrifuge-Outfitted with special carriers that accept deep 96-well plates, this machine is used for isolating DNA from tissue samples in a 96-well format that is amenable to multi-channel pipetting. The format increases the speed with which we process new samples by at least a factor of three.

DNA analysis software-Designed specifically for the analysis of DNA, RNA, and protein molecules, we use this software package to predict restriction patterns that might reveal variation between individuals, to analyze potential PCR primers, to align DNA sequences, etc. We also have software for automated genotyping using the fluorescent instruments.

Electrophoresis chambers--We have custom designed electrophoresis trays that enable us to run 96 samples simultaneously on a single gel. The sample wells on the combs are spaced precisely to match the spacing of standard multi-channel pipettors.

g. References.

Aebersold, P. B., G. A. Winans, D. J. Teel, G. B. Milner, and F. M. Utter. 1987. Manual for starch gel electrophoresis: A method for the detection of genetic variation. NOAA Tech. Rep. 61:1-19.

Allendorf, F. W., and N. Ryman. 1987. Genetic management of hatchery stocks. *In* N. Ryman and F. Utter (editors), Population Genetics and Fishery Management, p. 141-159. Univ. Washington Press, Seattle.

Emlen, J. M. 1991. Heterosis and outbreeding depression: a multi-locus model and an application to salmon production. Fish. Res. 12:187-212.

Fraidenburg, M. E., and R. H. Lincoln. 1985. Wild chinook salmon management: an international conservation challenge. N. Am. J. Fish. Manage. 5:311-329.

Glass, B., and C. C. Li. 1953. The dynamics of racial intermixture--an analysis based on the American Negro. Am. J. Hum. Genet. 7:368-385.

Hill, W. G. 1981. Estimation of effective population size from data on linkage disequilibrium. Genet. Res. (Cambridge) 38: 209-216.

Hindar, K., N. Ryman, and F. Utter. 1991. Genetic effects of cultured fish on natural fish populations. Can. J. Fish. Aquat. Sci. 48:945-957.

Kincaid, H. L. 1983. Inbreeding in fish populations used for aquaculture. Aquaculture 33:215-227.

Krimbas, C. B. and S. Tsakas. 1971. The genetics of Dacus oleae. V. Changes of esterase polymorphism in a natural population following insecticide control--selection or drift? Evolution 25:454-460.

Lewontin, R. C., and J. Krakauer. 1973. Distribution of gene frequency as a test of the theory of the selective neutrality of protein polymorphisms. Genetics 74:175-195.

Matthews, G. M., and R. S. Waples. 1991. Status review for Snake River spring and summer chinook salmon. U.S. Dep. Commer., NOAA Tech. Memo. NMFS F/NWC-200, 75 p.

Miller, W. H., T. C. Coley, H. L. Burge, and T. T. Kisanuki. 1990. Analysis of past and present salmon and steelhead supplementation. Part I. Report to Bonneville Power Administration, Project 88-100.

Milner, G. B., D. J. Teel, F. M. Utter, and C. L. Burley. 1981. Columbia River stock identification study: validation of method. Ann. Rep. Res. NOAA, Northwest and Alaska Fisheries Center, Seattle, Washington, 35 p.

Moran, P., D. A. Dightman, L. K. Park. 1998. Nonelectrophoretic genotyping using allele-specific PCR and a dsDNA-specific dye. Biotechniques *accepted*.

Moran, P., D. A. Dightman, R. S. Waples, and L. K. Park. 1997. PCR-RFLP analysis reveals substantial population-level variation in the introns of Pacific salmon (*Oncorhynchus* spp.). Mol. Mar. Biol. Biotechnol. 6:318-330.

National Research Council (NRC). 1996. Upstream: Salmon and society in the Pacific Northwest. National Academy Press, Washington, D. C.

Nehlsen, W., J. E. Williams, and J. A. Lichatowich. 1991. Pacific salmon at the crossroads: stocks at risk from California, Oregon, Idaho, and Washington. Fisheries 16(2):4-21.

Nei, M. and F. Tajima. 1981. Genetic drift and estimation of effective population size. Genetics 98:625-640.

Olsen, J. B., J.K. Wenberg, and P. Bentzen. 1996. Semiautomated multilocus genotyping of Pacific salmon (*Oncorhynchus* spp.) using microsatellites. Mol. Mar. Biol. Biotechnol. 5:259-272.

Park, L. K. and P. Moran. 1994. Developments in molecular genetic techniques in fisheries. *In*, G. R. Carvalho and T. J. Pitcher (eds.), Molecular genetics in fisheries, Reviews in Fish Biology and Fisheries. 4:272-299.

Park, L. K., P. Moran, D. Nickerson. 1994. Application of the oligonucleotide ligation assay to the study of chinook salmon populations in the Snake River. *In* L. K. Park, P. Moran, and R. S. Waples (editors), Applications of DNA technology to the management of Pacific salmon, p. 91-97. U.S. Dep. Commer., NOAA Tech. Memo. NMFS-NWFSC-17, 178 p.

Thompson, E. A. 1973. The Icelandic admixture problem. Ann. Hum. Genet. 37:69-80.

Utter, F., P. Aebersold, and G. Winans. 1987. Interpreting genetic variation detected by electrophoresis. In N. Ryman and F. Utter (editors). Population Genetics and Fishery Management, p. 21-45. University of Washington Press, Seattle.

Waples, R. S. 1988. Estimation of allele frequencies at isoloci. Genetics 118:371-384

Waples, R. S. 1989. A generalized method for estimating effective population size from temporal changes in allele frequency. Genetics 121:379-391.

Waples, R. S. 1990. Conservation genetics of Pacific salmon. III. Estimating effective population size. J. Heredity 81:277-289.

Waples, R. S. 1991. Genetic methods for estimating the effective size of cetacean populations. Rep. int. Whal. Commn (special issue 13):279-300.

Waples, R. S., and P. E. Smouse. 1990. Evaluation of gametic disequilibrium analysis as a means of identifying mixtures of salmon populations. American Fisheries Society Symposium 7:439-458.

Section 8. Relationships to other projects

Experimental design for this study was coordinated with state and tribal biologists, and we work closely with these groups each year in planning and conducting sampling. To the extent possible, sampling is also coordinated with other studies to minimize disturbance to the natural populations and maximize usefulness of the genetic and biological information collected. The following are the major related projects we have coordinated with for planning, sampling and/or dissemination of results.

Northeast Oregon: Early life history study of Grande Ronde Basin chinook salmon (Project 92-26-1, ODFW); Smolt migration characteristics and parr-to-smolt survival of naturally produced spring chinook salmon in the Grande Ronde and Imnaha river basins (part of Fish Passage Center smolt monitoring program); Evaluation of reestablishing natural production of spring chinook salmon in Lookingglass Creek, Oregon, using a non-endemic hatchery stock (CTUIR and ODFW, funded through LSRCP); Evaluation of the Lower Snake River Compensation Plan in Oregon (ODFW, funded through LSRCP); Grande Ronde spring chinook captive broodstock collection and maintanence (Projects 9606700, 9604400, and 9801001 funded through ODFW, NPT, LSRCP and NMFS).

Idaho:

Performance/stock productivity impacts of hatchery supplementation (Project 90-052, NBS); Idaho natural production and evaluation, Intensive monitoring subproject (Project 91-073, IDFG); Idaho supplementation studies (Project 89-098, IDFG); Monitoring the migrations of wild Snake River spring/summer chinook salmon smolts (Project 91-028, NMFS).

In addition, for the past several years we have shared juvenile chinook salmon collected under our study with Drs. Diane Elliot and Ron Pascho of the National Biological Service, who use the samples for analysis of bacterial kidney disease in their study, "Juvenile fish transportation: Impact of bacterial kidney disease on survival of spring/summer chinook salmon stocks," funded by the US Army COE. This collaboration was temporarily suspended because only fin clips were collected in 1995 and 1996 but may resume if adult returns improve.

Section 9. Key personnel

Robin S. Waples, Acting Director, Conservation Biology Division

B.A. in American Studies, Yale University, 1969.

Ph.D. in Marine Biology, Scripps Institution of Oceanography, 1986.

1986-present, research geneticist or fishery biologist at the Northwest Fisheries Science Center in Seattle

FTE commitment for FY1999 = 0.2

Dr. Waples developed this study and has been principal investigator since its inception. He has six years of experience in field collections of chinook salmon and steelhead for this study and is familiar with all the geographic areas and most of the local biologists. His research background is in the population genetics of fish, and he has published widely on topics such as the analysis of temporal genetic changes, hatchery-wild genetic interactions, genetic methods for estimating effective population size, and identification of conservation units for salmon under the ESA.

Many of the statistical analyses of the genetic data use techniques he developed several years ago for the study of temporal genetic variation in Pacific salmon.

Five relevant publications:

Waples, R. S. 1989. A generalized approach for estimating effective population size from temporal changes in allele frequency. Genetics 121:379-391.

Waples, R. S., and D. J. Teel. 1990. Conservation genetics of Pacific salmon. I. Temporal changes in allele frequency. Conserv. Biol. 4:144-156.

Matthews, G. M., and R. S. Waples. 1991. Status review for Snake River spring and summer chinook salmon. U.S. Dep. Commer., NOAA Tech. Memo. NMFS F/NWC-200, 75 p.

Waples, R. S., and C. Do. 1994. Genetic risk associated with supplementation of Pacific salmonids: Captive broodstock programs. Can. J. Fish. Aquat. Sci. 51 (Suppl. 1):310-329.

Allendorf, F. W., and R. S. Waples. 1996. Conservation and genetics of salmonid fishes. Pages 238-280 *in*: J. C. Avise and J. L. Hamrick, eds. Conservation genetics: Case histories from nature. Chapman and Hall, New York.

Paul B. Aebersold, Biological Technician

A.A.A. (1977) Marine Biology Technology, Shoreline Community College. National Marine Fisheries Service, Conservation Biology Division (1978 - present). Manager of the protein electrophoresis laboratory, collects genetic data and prepares for final analysis.

FTE commitment for FY1999 = 0.7

Paul Aebersold has worked as a Biological Technician for the National Marine Fisheries Service for almost 20 years. His responsibilities include overseeing and supervising the daily operation of the laboratory; scheduling the laboratory work to be done; interpreting the electrophoretic results; confirming the data being collected; preparing the data for statistical analyses; training new laboratory technicians or visiting scientists in protein electrophoresis techniques and in the interpretation of the results; assisting in the writing of reports and/or manuscripts; and leading the research effort in pursuing new techniques and new technologies which may improve our ability to meet our objectives. Mr. Aebersold also has over 10 years experience in the use of microcomputers to manage large genetic datasets, as well as 5 years of experience in basic network administration.

Publications

Aebersold, P. B., G. A. Winans, D. J. Teel, G. B. Milner, and F. M. Utter. 1987. Manual for starch gel electrophoresis: a method for the detection of genetic variation. U.S. Department of Commerce, NOAA Technical Report NMFS 61, 19 p.

Waples, R. S., O. W. Johnson, P. B. Aebersold, C. K. Shiflett, D. M. VanDoornik, D. J. Teel, and A. E. Cook. 1993. A genetic monitoring and evaluation program for supplemented populations of salmon and steelhead in the Snake River Basin. Report to Bonneville Power Administration, Contract No. DE-AI79-89BP00911.

Winans, G. A., P. B. Aebersold, and R. S. Waples. 1996. Allozyme variability of *Oncorhynchus nerka* in the Pacific northwest, with special consideration to populations of Redfish Lake, Idaho. Transactions of the American Fisheries Society 125:645-663.

Waples, R. S., P. B. Aebersold, and G. A. Winans. 1997. Population genetic structure and life history variability in *Oncorhynchus nerka* from the Snake River Basin. Report to Bonneville Power Administration, Contract No. DE-A179-93BP05326.

Winans, G. A., P. B. Aebersold, Y. Ishida, and S. Urawa. In press. Estimates of stock composition of chum salmon in highseas test fisheries in the western north Pacific Ocean and Bering Sea. Proceedings of the North Pacific Anadromous Fish Commission (NPAFC), October, 1996. Sapporo, Japan.

Paul Moran, Research Population Molecular Geneticist

B.S., in Biology, Southern Oregon State College (1985)

M.S., in Biology, Central Washington University (1988)

Ph.D., in Zoology, University of Maine (1993)

NMFS, NW Fisheries Science Center, Conservation Biology, Genetics, (1992 - present).

FTE commitment for FY1999 = 0.6

Dr. Moran directs laboratory research in the development of DNA markers and population genetic data collection and analysis. Research interests and activities include molecular phylogeography, population genetics, and changes in the genetic structure of populations over various temporal scales. Duties include supervision of students and technicians, as well as safety and environmental compliance responsibilities for the laboratory. Dr. Moran has over 10 years of experience in molecular genetics research, including evolutionary biology, systematics and population genetics. He is competent with commonly used computer programs for analysis of population genetic data (e.g., Arlequin, Biosys, Genpop, NTSYS, Popgene, Winamova). For the past 5 years, Dr. Moran has been involved in the development of PCR-RFLP and microsatellite markers in Pacific salmon. He has also developed allele-specific PCR and ligation capture assays. He is trained in hazardous materials emergency response, laboratory safety, environmental compliance, and hazardous materials shipping regulations. Dr. Moran also has considerable experience in field collection and PIT-tagging of salmonids, including snorkling, electrofishing, and seine netting.

Publications

Moran, P., D. A. Dightman, L. K. Park. 1998. Nonelectrophoretic genotyping using allele-specific PCR and a dsDNA-specific dye. Biotechniques *accepted*.

Moran, P., D. A. Dightman, R. S. Waples, and L. K. Park. 1997. PCR-RFLP analysis reveals substantial population-level variation in the introns of Pacific salmon (*Oncorhynchus* spp.). Mol. Mar. Biol. Biotechnol. 6:318-330.

Park, L. K. and P. Moran. 1994. Developments in molecular genetic techniques in fisheries. *In*, G. R. Carvalho and T. J. Pitcher (eds.), Molecular genetics in fisheries, Reviews in Fish Biology and Fisheries. 4:272-299.

Moran, P. and E. Bermingham. 1994. The phylogeographic structure of coho salmon (*Oncorhynchus kisutch*) populations assessed by mitochondrial DNA. *Abstract in*, L. K. Park, P. Moran, and R. S. Waples (eds.), Applications of DNA technology to the management of Pacific salmon, 57-62. U.S. Dep. Commer., NOAA Tech. Memo. NMFS-NWFS-17, 178 p.

Moran, P. 1994. Overview of commonly used DNA techniques. *In*, L. K. Park, P. Moran, and R. S. Waples (eds.), Applications of DNA technology to the management of Pacific salmon, p. 15-26. U.S. Dep. Commer., NOAA Tech. Memo. NMFS-NWFSC-17, 178 p.

Section 10. Information/technology transfer

We will continue to use a variety of methods to disseminate results of this research. Examples of reports and journal articles resulting from previous years of this project are listed elsewhere in this document. In 1993 we used funding from this project to host a workshop on application of DNA technology to the management of Pacific salmon and to publish the proceedings. Results of various aspects of this study have been presented in numberous public meetings throughout the region as well as nationally. In addition, we have provided many informal summaries of recent or unpublished results to fishery and hatchery managers in Washington, Idaho, and Oregon. These results have been used in an adaptive management framework to make real-time decisions about issues such as broodstock collection, mating protocols, and release strategies. Our efforts to develop RFLP loci are somewhat unique in salmon genetics and this work is beginnning to bear fruit both within the Columbia River and elsewhere. We have distributed PCR primers to many research groups in North America and Europe, and one of the